EpiTect® Plus FFPE Lysis Kit

The EpiTect Plus FFPE Lysis Kit included in the EpiTect Plus FFPE Bisulfite Kit (cat. no. 59144) can be stored at room temperature (15–25°C) for up to 6 months.

For more information, please refer to the *EpiTect Plus Bisulfite Conversion Handbook*, which can be found at www.qiagen.com/handbooks.

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

Notes before starting

- Prepare reagents as described in EpiTect Plus DNA Bisulfite Kit (included).
- Scrape any FFPE samples on slides into a 200 µl reaction tube or 8-well strip and proceed with step 1.
- Perform all centrifugation steps at room temperature (15–25°C).
- Do not place samples on ice after beginning the lysis procedure.

1. Place a FFPE slice in a 200 µl reaction tube or 8-well strip (not provided) and add 150 µl Deparaffinization Solution.
   The slice should be 10 µm thick, with a surface area ≤100 mm^2.
2. Flick or vortex the tube until all paraffin is dissolved.
3. Add 20 µl distilled water, 15 µl Lysis Buffer FTB, and 5 µl proteinase K.
4. Vortex and briefly centrifuge the samples.

   Note: The Deparaffinization Solution will form a layer above the Lysis Buffer FTB with the added proteinase K
5. Program a thermal cycler according to Table 1 for lysis and decrosslinking.
6. Place the PCR tubes with the lysis reactions into the thermal cycler and start the incubation. Proceed as soon as possible with bisulfite conversion.

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7. Add 800 µl RNase-free water to each aliquot of Bisulfite Mix needed, and vortex until Bisulfite Mix is completely dissolved. This can take up to 5 min.

   Note: Dissolving the Bisulfite Mix may require heating the solution to 60°C.

8. After the lysis incubation (step 6) is completed, remove the Deparaffinization Solution (approximately 130 µl) from each lysis reaction. Remove as much Deparaffinization Solution as possible without disturbing the lysed sample material to make sufficient space in the reaction tube for the bisulfite reaction components.

9. Set up the bisulfite reactions by adding to the lysis reaction the reagents listed in Table 2. Add each component in the order listed.

10. To perform the bisulfite DNA conversion, proceed to Protocol 1 "Bisulfite conversion of DNA" included in the supplied EpiTect Plus DNA Bisulfite Kit.

Table 1. Lysis thermal cycler conditions

Step	Time	Temperature
Lysis	30 min*	56°C
Decrosslinking	60 min	95°C

* Ensure that the tissue is completely lysed; if not, add an additional lysis step (30 min at 56°C).

Table 2. Bisulfite reaction setup

Component	Volume per reaction (µl)
Lysis reaction (step 8)	Approximately 40
Bisulfite Mix (dissolved), see step 7	85
DNA Protect Buffer	15
Total volume	**140**

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